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Specific Cesium Transport via the *Escherichia coli* Kup (TrkD) K^+ Uptake System

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***Escherichia coli* cells which contain a functional Kup (formerly TrkD) system took up Cs^+ with a moderate rate and affinity. Kup is a separate K^+ uptake system with relatively little discrimination in the transport of the cations K^+, Rb^+, and Cs^+. Regardless of the presence or absence of Kup, K^+-replete cells took up Cs^+ primarily by a very low affinity mode, proportional to the ratio of the Cs^+ and K^+ concentrations in the medium.**

One of the striking consequences of the Chernobyl reactor catastrophe is the prolonged occurrence of radioactive cesium (Cs^+) in animals, plants, and fungi. Higher animal cells show relatively little discrimination between the transport across the cytoplasmic membrane of K^+ (ionic radius, 0.133 nm) and the larger monovalent cations Rb^+ (radius, 0.148 nm) or Tl^+ (radius 0.143 nm) (5). The Na^+/K^+-ATPase catalyzes uptake of Cs^+ (radius, 0.169 nm) (24). By contrast, microorganisms and especially bacteria possess alkali cation transport systems that are much more selective for the different cations (13, 22). The constitutive K^+ uptake system Trk from *Escherichia coli* shows approximately a 10-fold discrimination between K^+ and Rb^+ or Tl^+ (1, 8, 20), and the inducible, high-affinity K^+ uptake ATPase Kdp is even more selective (8, 20). Since very little is known about Cs^+ accumulation by microorganisms besides what is in a few reports (7, 15) and since knowledge about such accumulation is essential for the estimation of the extent to which microorganisms may contribute to the prolonged retention of radioactive Cs^+ by soil, we determined which *E. coli* K^+ uptake systems accept Cs^+. This is the only microorganism for which a number of well-defined K^+ transport mutants are available (10, 19, 23). We report here that Cs^+ is only taken up via the TrkD system. This observation supports the original but later abandoned view (cf. reference 19 with references 11 and 23) that the TrkD system is a separate K^+ uptake system. It possesses a low specificity for the alkali cations transported and is thereby clearly distinct from the two main K^+ uptake systems Trk and Kdp. To avoid future confusion with the Trk system we will refer to the TrkD system, which is the product of the *trkD* gene(s), as Kup (mnemonic for K^+ uptake).

Bacterial strains and growth conditions. All of the strains used are derivatives of *E. coli* K-12 kindly provided by W. Epstein, The University of Chicago, Chicago, Ill. The genotypes of these strains and the K^+ uptake systems expressed under the different growth conditions are listed in Table 1. The growth media were those described by Epstein and Kim (10).

Plasmid pJG1. The *trkD*-containing plasmid pJG1, described by Lopilato et al. (16), was obtained from W. Epstein. It consists of a 6.3-kilobase *Eco*RI-*Hin*dIII fragment cloned into the large *Eco*RI-*Hin*dIII fragment of pBR322 and contains the *trkD* gene (D. C. Dosch, Ph.D. thesis, The University of Chicago, Chicago, Ill. 1985), as well as the *rbsA* gene (14).

K^+ depletion and transport assays. Growing cells were harvested by centrifugation after they had reached an optical density at 578 nm between 0.7 and 1.0. These cells were depleted of K^+ by Tris-EDTA treatment as described previously (4). Transport assays were carried out at 23 to 25°C with EDTA-treated cells suspended at 1 mg (dry wt) ml of medium^{-1} consisting of 200 mM sodium HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.5) and 10 mM glucose (4). After a preincubation of 15 min, uptake assays were started by the addition of KCl, $^{86}RbCl$, or CsCl at the concentration indicated at the particular experiment. Cells from 1.0-ml samples were centrifuged through silicone oil. The K^+ and Cs^+ contents of the cell pellets were determined in a flame-photometer 700 (Eppendorf Geratebau, Hamburg, Federal Republic of Germany) and at 852.1 nm in the emission mode of an atomic absorption photometer 357 (Instrumentation Laboratory Inc., Lexington, Mass.), respectively. The $^{86}Rb^+$ content of the cell pellets was determined in a 460C-liquid scintillation counter (Canberra-Packard, Frankfurt, Federal Republic of Germany).

Cs^+ uptake by K^+ uptake mutants. Figure 1 gives rates with which K^+-depleted cells of the different K^+ transport mutants showed net Cs^+ uptake. Strains that possess a functional Kup system (i.e., FRAG-1, FRAG-5, or TK1001 [Fig. 1, closed symbols]) took up Cs^+ fairly rapidly (V_{max}, 15 to 25 μmol min^{-1} g [dry wt] of cells^{-1}; K_m, 5 to 7 mM). Strains that only possess a Kdp system (strains TK2240 or TK2242) or Trk system (TK1001) took up Cs^+ as slowly as did strain TK2205, which is deleted for the Kdp system and carries point mutations in both the *trkA* and *trkD* genes (Fig. 1, open symbols).

Enhanced Cs^+ uptake by cells carrying the *trkD* gene on a multicopy plasmid. Cells that were either *kdp trkA trkD*, *kdp trkD trkE trkG*, or *kdp trkD trkG trkH* and contained the *trkD* gene on the multicopy plasmid pJG1 (16) took up K^+ several times faster than did cells that carried a functional *trkD* gene in the chromosome (D. Bossemeyer, I. R. Booth, and E. P. Bakker, Short Rep. Fifth Eur. Bioenergetics Conf. Aberystwyth, Wales, 1988, p. 199). The same was true for Cs^+ uptake (Fig. 1). Since Cs^+ is only taken up via the Kup system (see above), these results suggest that the *E. coli* chromosomal DNA of plasmid pJG1 alone is sufficient to encode a functional Kup system.

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TABLE 1. K^+ uptake mutants of *E. coli* K-12 and their properties[a]

Strain	Genotype[b]	[K^+] during growth	K^+ transport systems expressed
FRAG-1	*gal*	30 mM	Trk, Kup
FRAG-5	*kdpABC5 gal*	5 mM	Trk, Kup
TK1001	*kdpABC5 trkD1 gal*	5 mM	Trk
TK1110	*kdpABC5 trkA405 gal*	5 mM	Kup
TK2205	*kdpABC5 trkD1 trkA405 gal nagA*	115 mM	None[c]
TK2240	*trkA405 trkD1 nagA*	100 μM[d]	Kdp
TK2242	*kdp-42 trkA405 trkD1 nagA*	5 mM	Kdp*[e]

[a] Genetic data are from references 10, 12, and 19.
[b] All of the strains are also F^- *lacZ rha thi*.
[c] The slow K^+ uptake observed with this strain may be mediated by a fourth uptake system, TrkF (19).
[d] Cells of strain TK2240 were grown overnight at 0.1 mM K^+. On the next day, they were diluted 1:20 into fresh medium without added K^+, but which was contaminated with about 25 μM K^+. After growth had ceased, 25 μM KCl was added to the suspension. After growth had ceased again, K^+ was added at 100 μM. The cells were harvested after growth had resumed.
[e] The Kdp* system possesses a greatly diminished affinity for K^+ (12).

Ion specificity of the Kup system. That the Kup system accepts Cs^+ suggests that it does not discriminate strongly between K^+ and Cs^+. This differs from previous findings that the TrkD system distinguishes even more strongly between K^+ and Rb^+ than does the Trk system (20). We cannot confirm this earlier result. First, the kinetic parameters of K^+ and Rb^+ via Kup were almost identical (Table 2). Second, the uptake of tracer amounts of $^{86}Rb^+$ by the cells in the presence of large amounts of either KCl or RbCl indicate that ^{86}Rb uptake mimics bulk K^+ uptake by the Kup system very well (results not shown). Table 2 also shows a large difference between the transport of K^+ and Rb^+ via the Kup system and that of Cs^+ in the approximately 10-fold-higher K_m value of the latter. Thus, in contrast to Trk or Kdp, the Kup system does not strongly distinguish between the alkali cations K^+, Rb^+, or even Cs^+ (cf. Fig. 1 and Table 2 with references 1 and 20).

Inhibition of K^+ uptake by Cs^+. Even a compound not translocated by a transport system may still inhibit the transport of the natural substrate. This effect was very small for Cs^+ acting on K^+ uptake by the Kdp system. A 30 mM concentration of Cs^+ inhibited the net uptake of K^+ (added at 2 mM) via the altered Kdp system (Table 1) of strain TK2242 by less than 10%. The effect of Cs^+ on K^+ uptake via the Trk system was larger with competitive inhibition and a K_i of 30 mM Cs^+. As expected, the strongest effect of Cs^+ was observed on K^+ uptake via the Kup system. The inhibition was competitive with a K_i of 7 mM Cs^+ (results not shown).

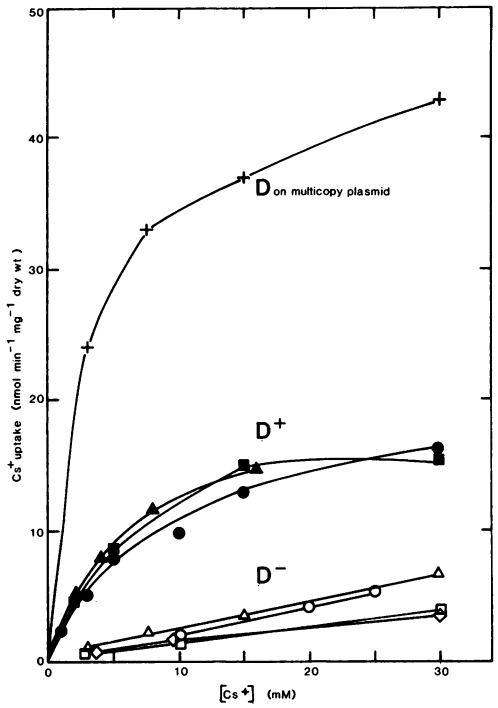

FIG. 1. Cesium uptake by the Kup system. Cells of the *E. coli* K^+ uptake mutants were tested for Cs^+ uptake. Symbols: +, strain TK2205 containing the *trkD* gene on multicopy plasmid pJG1; ■, FRAG-1; ●, FRAG-5; ▲, TK1110; △, TK1001; ○, TK2205; □, TK2240; ◇, TK2242.

Cs^+ uptake by K^+-loaded cells. Both Kdp- and Trk-like bacterial K^+ uptake systems shut off their activity when the cells become loaded with K^+ (2, 17, 18). This effect is due to an increase in cell turgor during net K^+ uptake (6, 9), which occurs because in this process K^+ is partially exchanging against protons (3, 21). The decrease of Trk activity in K^+-loaded cells led to a remarkable effect when K^+ and an excess of Cs^+ were added simultaneously to K^+-depleted cells of either strain FRAG-5, which contains functional Trk and Kup systems (Fig. 2A), or TK1001, which only possesses a Trk system (Fig. 2B). In both situations, the cells were first rapidly loaded with K^+. However, subsequently the cells slowly exchanged K^+ for Cs^+. From the exchange rate in strain TK1001, it can be calculated that in K^+-loaded cells the activity of the Trk system was as low as 3 μmol min^{-1} g^{-1}, which is about 1 to 2% of the V_{max} for K^+ uptake by K^+-depleted cells (1, 19). Hence, the effect of shutting off Trk activity during loading of the cells with K^+ is much larger than has been recognized before (17, 18). However,

TABLE 2. Cation specificity of *E. coli* K^+ uptake systems

System[a]	K^+ uptake[b]		Rb^+ uptake[b]		Cs^+ uptake[b]		References
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	
Kdp	0.002	150	Low activity	Low activity	ND[c]	ND	19, 20, this work
Trk	0.9–1.5	190–500	0.35	18	ND	ND	1, 19, this work
Kup	0.37 ± 0.13[d]	27 ± 5[d]	0.38 ± 0.07[e]	38 ± 1[e]	5 ± 1[e]	17 ± 3[e]	This work

[a] Data from strains TK2240, TK1001, and TK1110 for the Kdp, Trk, and Kup systems, respectively.
[b] K_m in millimolar; V_{max} in micromoles $minute^{-1}$ $gram^{-1}$ (dry wt) of cells.
[c] ND, No activity detected.
[d] Average from four experiments with standard deviations.
[e] Average from two experiments with standard deviations.

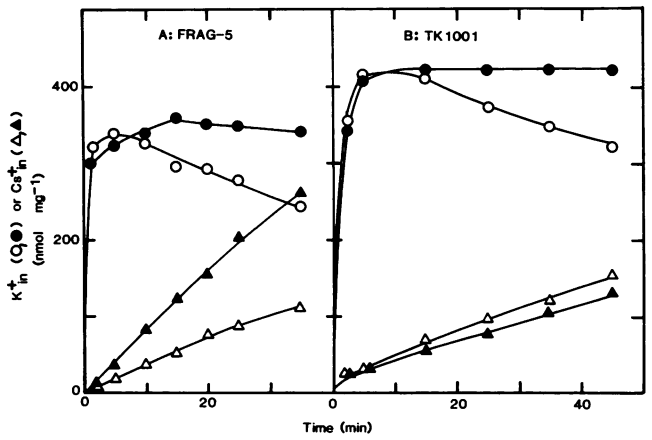

FIG. 2. K^+-Cs^+ exchange by *E. coli*. To glucose-metabolizing, K^+-depleted cells of strain FRAG-5 (A) or TK1001 (B) were added, at zero time, either 5 mM KCl (●), 30 mM CsCl (▲) or 5 mM KCl plus 30 mM CsCl (○, △); circles, K^+ content; triangles, Cs^+ content.

Mulder (M. M. Mulder, Ph.D. thesis, University of Amsterdam, Amsterdam, The Netherlands, 1988) drew a conclusion similar to ours.

Very slow Cs^+ uptake. Cells that do not possess a functional Kup system took up Cs^+ very slowly (Fig. 1). This Cs^+ uptake nevertheless saturated at high Cs^+ concentrations (V_{max}, 10 to 20 μmol min^{-1} g^{-1}; K_m, 100 to 150 mM; data not shown) and may either represent uptake via a fourth, very low affinity K^+ uptake system, TrkF (19), or reflect a leak current through the cytoplasmic membrane driven by the internally negative membrane potential (19, 23). The second point illustrated by Fig. 2 is that in the presence of both K^+ and Cs^+, the uptake of Cs^+ by K^+-loaded cells occurred via this fourth system, since regardless of the presence or absence of a functional Kup system the two strains showed the same rate of Cs^+ uptake (Fig. 2, open triangles). Since this system also shows almost no discrimination between K^+ and Cs^+ (data not shown), the extent of accumulation of radioactive Cs^+ by wild-type, K^+-replete *E. coli* should be proportional to the ratio of the Cs^+ and K^+ concentrations present in the medium.

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